

Mercury Analysis of Body Lotion Cosmetic Using CVAAS Method: Case Study of Distributed Product in Banda Aceh

Eka Safitri¹, Irmawati Irmawati¹, Khairi Suhud^{1*}, Nurul Islami²

¹Chemistry Department, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh.

²Mathematics Department, STKIP Bina Bangsa Getsempena, Banda Aceh

*Corresponding Author: khairi@unsyiah.ac.id

Abstract

The aim of this work is to determine the concentration of mercury in body lotion from several brands that are available in Banda Aceh. This research expected to provide information for the public and government related to the negative effects of harmful cosmetics that widely circulated. This information is useful into consideration of policy-making and monitoring the use of Hg metal. This study determined the type of body lotion cosmetics containing Hg metal by using Cold Vapor Atomic Absorption Spectroscopy (CVAAS) method. There are 22 samples and 11 of them have been selected by purposive sampling. The standard Hg calibration curve produces a linear line with the equation $y = 0.0874x + 0.0729$, and the correlation coefficient (R^2) value of 0.9726. LOD value is 0.004854 ppb, and LOQ is 0.01681 ppb. The positive results were obtained from samples code TB3= 0.046, TB6= 0.01, and TB13= 0.004 ppb respectively. These results indicated the mercury level was still lower than 1 mg/L the threshold by drug and food control centers (BPOM).

Keywords: Mercury, CVAAS, Body Lotion, Cosmetic Product, Wet Destruction, Banda Aceh

Abstrak (Indonesian)

Riset dilaksanakan untuk menentukan konsentrasi merkuri yang terdapat dalam produk kosmetik yang tersebar di Banda Aceh. Penelitian ini diharapkan memberi informasi bagi masyarakat dan pemerintah tentang dampak negatif kosmetik yang beredar secara bebas. Informasi tersebut selanjutnya dapat dijadikan bahan pertimbangan dalam pengambilan kebijakan dan melakukan pengawasan dalam penggunaan logam Hg. Penelitian ini bertujuan untuk mengetahui sediaan jenis kosmetik *body lotion* yang mengandung logam Hg di kota Banda Aceh, dan menentukan kadar logam Hg yang terkandung di dalam kosmetik dengan menggunakan metode Vapour Atomic Absorption Spectroscopy (CVAAS). Populasi merkuri dalam *Body Lotion* ada 22 dan yang sampel yang dianalisis sebanyak 11. Kurva kalibrasi standar Hg menghasilkan garis linier dengan persamaan $y = 0,0874x + 0,0729$, beserta nilai koefisien korelasi (R^2) adalah 0,9726. Nilai LOD adalah 0,004854 ppb, dan LOQ adalah 0,01681 ppb. Hasil Positif dari sampel ini didapat dari sampel dengan kode TB3= 0,046, TB6= 0.01, dan TB13= 0,004 ppb. Hasil ini menunjukkan bahwa kadar merkuri masih di bawah standar yang ditentukan oleh Badan Pengawas Obat dan Makanan Indonesia (BPOM) dengan nilai di bawah 1 mg/L.

Kata Kunci: Mercury, CVAAS, Body Lotion, Cosmetic Product, Wet Destruction, Banda Aceh

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INTRODUCTION

Heavy metals are metals that have a specific gravity of 5.0 cm³ or more, and based on the toxicological point of view there are essential and non-essential heavy metals. Essential heavy metals in certain amounts are needed by living organisms, but in

excessive amounts will turn into poisons. Essential heavy metals are Zn, Cu, Fe, Co, Mn, and Se, and non-essential heavy metals are toxic metals and can cause various adverse effects on living things and cannot be decomposed. Examples of non-essential metals As, Cr, Sn, Pb, Cd, and Hg (Mercury) [1].

Mercury is a poisonous element found in the earth's crust. In nature Hg is usually in the form of organic compounds such as methyl mercury and inorganic salts of mercury chloride. The toxicological effects of Hg on body organs are like those of the lungs, kidneys, and skin. The element Hg is a liquid metal at room temperature, volatile, can conduct electricity, and can form alloys or amalgams. Mercury is widely applied in the extraction of chemicals, the process of making drugs, basic ingredients of insecticides, and cosmetics [2].

Skin lightening cosmetic products such as gels, whitening creams, body lotions, and soaps are reported to contain highly toxic active ingredients in the form of hydroquinone and Hg [3]. The beauty product is intentionally added to the Hg metal which can inhibit the formation of melanin. Melanin occurs in intracellular ovoid organelles known as melanosomes, and is produced by dendritic melanocytes that are found in epidermal cells by 2% [4].

Mercury exposure in the short term can cause nausea, coughing, shortness of breath, and sore throat. Whereas in the long run it can cause anxiety, tremors, impairing hearing or hearing, and loss of appetite. Hg metal if entered into the digestive tract will be absorbed by epithelial cells and can cause digestive disorders by preventing the synthesis and secretion of the digestive enzymes of trypsin, chymotrypsin, and pepsin, as well as disrupt the function of xanthine oxidase. At the cellular level, mercury causes changes in the structure of macromolecules and membrane permeability [5].

The use of cosmetics can be detrimental if excessive, errors in processing, improper use of materials, and unhygienic storage. Skin reaction to cosmetics will cause irritation, burning sensation, sore, or sometimes the surface is runny. Errors in the use of cosmetics can also cause photosynthetic reactions such as allergies when exposed to the sun. The abnormality can be in the form of itching, red spots, and being blackish or called hyperpigmentation [6].

According to the regulation of the head of the Republic of Indonesia drug and food control regulatory agency (BPOM RI) number 17 of 2014 concerning the requirements of microbial and heavy metal contamination in cosmetics that the type of Hg contamination is not more than 1 mg/l (1 ppm). Rumondang & Lestari reports that mercury levels in some body lotions are 0.003 ppm with the detection of mercury analyzer [7]. Furthermore, it was reported by Sari et. al, [8] that a qualitative analysis of body lotions sold online positively contained Hg. Therefore, we are

interested in examining Hg levels in body lotion products.

The sampling technique uses purposive sampling technique, where the sample is grouped into two parts. The first sample has a list of permits from BPOM and the second sample is not having a BPOM permit. Samples are selected based on the most widely used (branded) and non-branded. Next, the samples were prepared by the wet destruction method, and analyzed using detection of Cold Vapor Atomic Absorption Spectroscopy (CVAAS).

AAS instrument is a tool used in the analysis method of determining the elements of metals and metalloids based on the absorption of radiation by free atoms. The working principle of the AAS method is that the absorbance is directly proportional to the length of the flame through which the light and the vapor concentration of the atom are in the flame [9]. This research will analyze the Hg metal content in body lotion cosmetics of various brands circulating around the city of Banda Aceh.

This study to determine the type of body lotion cosmetics containing Hg metal that distributed in the Banda Aceh. This Study also to determine the Hg metal content contained in cosmetics by using the AAS method. This research is expected to provide information for the public and government about the negative effects of harmful cosmetics that circulate freely. This information can then be taken into consideration in policy-making and monitoring the use of Hg metal.

MATERIALS AND METHODS

Tools and Materials

The tools used in this study are CV AAS tools, analytical scales, a set of wet destruction equipment, analytical scales, Beaker cups, measuring cups, drop pipettes, petri dishes, and volume pipettes, ice baths, and condensers. The materials used in this study were body lotion, aquadest, H₂SO₄, HNO₃, HClO₄, HgCl₂, NaOH (all the reagents form and aquadest Merck).

Sampling

The sample used in this study was 11 products from 22 body lotion products of various brands taken around the city of Banda Aceh. Samples taken in this study are branded and non-branded, then samples that have BPOM permission and do not have BPOM permission. The sample chosen was purposive sampling, which is a sample that is widely used by consumers, along with samples that promise white skin in a relatively fast time span. Then the samples are

grouped and analyzed. Sample preparation uses the wet destruction method and Hg content is analyzed using CV AAS, and the details can be seen in Table 1.

Table 1. The sample used for research

Number	Sample Code	Inspection Remarks
1.	TB ₁	BPOM
2.	TB ₂	BPOM
3.	TB ₃	BPOM
4.	TB ₄	BPOM
5.	TB ₆	-
6.	TB ₉	BPOM
7.	TB ₁₀	BPOM
8.	TB ₁₃	-
9.	B ₁	BPOM
10.	B ₂	BPOM
11.	B ₈	BPOM

TB: non-Branded

B: Branded

Methods

Mercury Stock Solution Procedure

Metal analysis is carried out based on the SNI 6989.6: 2009 method [10]. HgCl₂ powder was weighed as much as 0.0135 grams and diluted with distilled water in a 100 mL measuring flask so that a stock solution of 100 ppm was obtained. Then pipette 1 mL and diluted with distilled water in a 1000 mL measuring flask to obtain a solution of 1 ppm Hg metal ion stock, then pipette 0.1 mL and diluted in a 100 mL measuring flask and obtained a standard solution of 100 ppb [10].

Making a Standard Solution

The solution of 100 ppb Hg metal ion stock was pipetted as much as 10 mL and diluted with distilled water in a 100 mL measuring flask, so that a solution of 10 ppb Hg metal ion stock was obtained. Furthermore, pipette 0.25; 0.5; 0.75 and 1 mL to be diluted 100 mL so that the concentration becomes 0.25; 0.5; 0.75; and 1 ppb to be analyzed into a CV AAS tool at a wavelength of 253.7 nm [10].

Destruction Process

The sample was weighed as much as 5 grams and put into a 500 mL round bottom flask, added 10 mL of concentrated HNO₃, 15 mL concentrated HClO₄, 20 mL concentrated H₂SO₄ slowly through the pumpkin wall and 5 mL aquadest. Then, the pumpkin is connected to the cooler. After that, the sample is heated on a bath at 100 °C for 2 hours until the solution becomes clear and a sample solution of 50 mL is obtained. The digestion process was repeated three times so that the total sample volume was 150 mL. Then the sample is allowed to cool at room

temperature, and stored in an Erlenmeyer glass and covered with aluminum foil. Samples can be used for qualitative and quantitative analysis [11].

Qualitative Analysis

Destructive samples were analyzed qualitatively by using NaOH 2 N reagent to determine the metal content of Hg in body lotion. A sample solution of 1-2 mL pipetted is inserted into a test tube, added 1-2 drops of NaOH solution, if a yellow precipitate is formed then a positive sample contains Hg metal in the form of mercury oxide (II) [8].

Data Analysis and Validation

Precision Test Equipment

Hg standard solution with a concentration of 7.5 ppb was analyzed using AAS at a wavelength of 253.7 nm four times. The absorbance results are used to calculate the average absorbance, the standard deviation (SD) value, the relative standard deviation (RSD), and the accuracy of the tool using the following equation.

$$\bar{x} = \frac{x_1 + x_2 + x_3 + \dots + x_n}{n} \quad (1)$$

$$SD = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n - 1}} \quad (2)$$

$$\%RSD = \frac{SD}{\bar{x}} \times 100\% \quad (3)$$

$$\text{Accuracy of the tool} = 100\% - \frac{SD}{\bar{x}} \quad (4)$$

Linearity Test

Linearity test using standard mercury standard curve solutions that have been obtained, the absorbance results obtained are used to calculate the value of the correlation coefficient (r), slope (slope), and the value of the intercept. Linearity of a method can be seen from the response of measurement results with concentrations approaching a straight line with the following equation.

$$y = ax + b \quad (5)$$

with y: instrument response, x: analyte levels, a: slope, and b: intercept

Detection of Limits (LOD)

Detection limits can be calculated based on blank absorption measured four times and standard deviation (SD) blank. The equation used to calculate the detection limit is as follows.

$$LOD = \frac{3 SD}{Slope} \quad (6)$$

$$SD = \sqrt{\frac{\sum(x_1 - \bar{x})}{n - 1}} \quad (7)$$

with LOD: detection limit, SD: standard deviation, n: total data and *Slope*: slope

Limit of Quantitation (LOQ)

Limit of Quantitation (LOQ) is the Quantitation limit of Hg 0.5 ($\mu\text{g} / \text{g}$), if the test results are below the specified quantitation limit, then it is reported as below the quantitation limit. If the Hg metal content is high, the test solution must be diluted to levels within the calibration curve range. Dilution factors are needed if the sample is further diluted [12]. The equation used to calculate LOQ is as follows.

$$LOQ = \frac{10 SD}{Slope} \quad (8)$$

With LOQ: limit of quantitation, SD: standard deviation and *Slope*: slope.

Hg level test

The extracted samples were analyzed using CV AAS with a wavelength of 253.7 nm. Furthermore, absorbance results are included in the standard curve that has been obtained [13].

RESULTS AND DISCUSSION

Sample Preparation

A total of 11 body lotion samples from 22 samples were selected by purposive sampling, structured with the aim of changing the sample of organic compounds into pure elements. The digestion process in this study uses a mixture of strong acid solutions such as HNO_3 , HClO_4 , H_2SO_4 , and aquadest which function as oxidizing agents. The wet destruction method is carried out with the help of heat which aims to accelerate the oxidation and reshuffle processes as well as termination between organic compounds and Hg metals in body lotion samples

The destruction process is carried out at a temperature of 100 °C, the destruction is carried out on a bath in a fume hood so that the steam produced directly goes out into the free air and does not poison the surrounding environment. Eleven prepared samples have different destructive time spans, the average sample being able to be destroyed for 2 to 5 hours. Some samples that are difficult to experience an overhaul will be destroyed for 11 hours (TB7, TB8, TB10 and TB13), because the texture of the sample is very dense. Destruction was carried out three times over to get 150 mL which was used as a qualitative and quantitative analysis.



Figure 1. Wet destruction process and the result

The combination of concentrated acid as an oxidizing agent can easily release hydrogen ions so that the bonding between the metal and the organic compound occurs which is characterized by the formation of a reddish brown form of NO_2 gas. Wet destruction was chosen because Hg is volatile, and the temperature used is relatively lower so as to minimize the occurrence of evaporation of Hg in the sample. In addition, the use of a condenser serves to reduce analytic loss due to evaporation during the sample destruction process. The perfection of destruction is characterized by the formation of a clear solution, indicating that the constituents contained in the sample are completely dissolved or the overhaul of the organic compound is going well [14].

Qualitative Analysis of Hg

Qualitative analysis aims to determine the presence of Hg ions contained in the sample. Qualitative test results with color reagents using 2 N NaOH solution did not indicate that a positive sample contained Hg. Qualitative analysis cannot detect Hg in low concentrations. Hg levels in body lotion samples have very low so quantitative analysis is needed that is able to detect Hg at low concentrations.

The precision test was carried out using a standard solution of 0.4 ppb Hg with four iterations. The precision test was carried out using CV AAS at a wavelength of 253.7 nm. A precision test is carried out for the accuracy of an instrument based on the level of accuracy of the individual analysis results indicated by the price of SD and RSD. The precision test results can be seen in Table 2.

The measurement results show, SD and % RSD are 2% ie 1.829%. The price of SD and % RSD generated is good, because the % RSD generated is smaller than 5%. Galat are errors in measurement, then:

$$\begin{aligned} \text{Error} &= \text{true value} - \text{value obtained} \\ &= 0.4 - 0.397 \end{aligned}$$

= 0.0027

The error obtained is 0.0027, and the value obtained is quite small.

Table 2. Tool precision test results

No	Hg Standards (ppb)	Absorbance (A)
1	Repetition 1	0.3974
2	Repetition 2	0.3974
3	Repetition 3	0.3973
4	Repetition 4	0.3971
5	Average absorbance	0.3973
6	SD	0.727
7	%RSD	1.829
8	Accuracy of the tool	0.829

The accuracy test performed to show the degree of closeness of the results of the analysis with the actual level of the analyte. Accuracy is expressed as a percent of recovery. Accuracy test is performed with the levels of the results of the analysis divided by the actual levels. The accuracy of the analysis results depends on the errors in the entire analysis phase. Recovery value is obtained at 99.33% which meets the requirements of good accuracy from 90-110%.

In this research, Linearity test to measure the calibration curve connects the response (y) with concentration (x), linearity is measured by a single measurement at different concentrations. Linearity test is done by determining the straight-line equation between concentrations of changes in absorbance using a standard Hg solution with a concentration of 0.25; 0.5; 0.75; and 1 ppb. The linearity test results obtained the calibration curve is $y = 0.087x + 0.009$ with a correlation coefficient (r) of 0.9726. Based on the results of the equation can be used as a basis for calculating the concentration (x) with absorbance values (y) as in Figure 2, the results of the linearity test can be seen in Table 3.

Table 3. Linearity test results

Concentration (ppb)	Absorbance (A)
0	0.002
0.2	0.0338
0.4	0.0436
0.6	0.0651
1	0.093

Slope = 0.0874
Intercept = 0.009
Correlation = 0.9726

The calibration curve is stating the relationship between absorbance (A) and concentration (ppb), then the linear region of the curve is determined to provide

a measurement limit (Makmun, 2013). Based on the calibration curve (Figure 2) it can be seen that the relationship between concentration and absorbance shows that the greater the concentration, the greater the absorbance (positive correlation). Hg standard calibration curve produces a linear line with the equation $y = 0.0874x + 0.0729$, along with the value of the correlation coefficient (R^2) is 0.9726. Calibration curves with correlation coefficients close to one indicate that the calibration curve is linear [15].

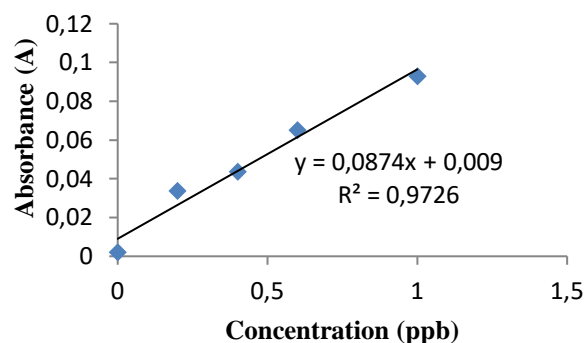


Figure 2. Hg standard calibration curve

The Limit of Detection (LOD) measurement is the smallest number of analytes in a sample that can be detected and gives a significant response compared to the blank. The SD blank value is 0,000141, and the LOD is $3 \text{ SD/slope} = 0.004854 \text{ ppb}$. Furthermore, the LOQ value is $10 \text{ SD/slope} = 0.01681 \text{ ppb}$. LOD and LOQ measurement results can be seen in Table 4. Based on the results obtained, the LOD value obtained is quite low and so is the LOQ value. This shows that the concentration of Hg in the sample can be measured because the LOD is lower than the sample concentration. Furthermore, the results of LOQ concentration are still below the quality standards set by BPOM (0.5 ppb).

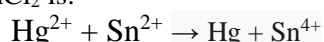
Table 4. LOD and LOQ blank

No	Blank	Absorbance (A)
1	Repetition 1	0.0016
2	Repetition 2	0.0019
3	Repetition 3	0.0016
4	Repetition 4	0.0017
5	Average absorbance	0.0017
6	SD	0.000141
7	LOD	0.004854 ppb
8	LOQ	0.01681 ppb

Quantitative Analysis of Hg

Quantitative test is done, because the qualitative test does not show the presence of Hg metal content in the sample. Quantitative test is done because the lotion sample used is thought to contain Hg content. Hg quantitative test using AAS combined with CV (Cold Vapor) techniques, namely the MVU (Mercury Vapor Unit). The CVAAS technique is the most used technique in determining Hg levels because the CV technique has accuracy, sensitivity, a high level of accuracy, and ability to analyze samples with low concentrations [16].

Quantitative analysis of Hg determination using the CV AAS technique was carried out with a flameless system. The sensitivity of this tool is quite good and does not interact with other metals in the sample. The working principle of flameless-AAS is the suction of liquid samples containing Hg^+ is carried out using a suction pipe connected to a peristaltic pump. In addition, the use of SnCl_2 reducing agent in an acidic atmosphere is able to reduce Hg^+ by about 95% so that the Hg in the sample is converted to a neutral gas-shaped Hg which is further excited by absorbing light from the Hg cathode lamp. Measurement of Hg levels using hollow Hg cathode lamps, so that each metal has the right level of excitation with a cathode lamp that can emit radiation energy in accordance with the energy required [17]. The reaction mechanism between Hg_2^+ and SnCl_2 is:



The quantitative sample test results showed that eleven samples analyzed with CVAAS only three samples (TB_3 , TB_6 , and TB_{13}) were positive for Hg, while the other eight samples were below the detection limit of the instrument $< 0,0001$. Sample levels containing Hg were: $\text{TB}_3 = 0.046$; $\text{TB}_6 = 0.001$ and $\text{TB}_{13} = 0.0040$ ppb. The TB_3 code sample has a BPOM permit, while the samples with the TB_6 and TB_{13} codes do not have a BPOM permit. The Hg level analyzed for the sample is still below the allowed quality standard, which is 1 ppm [12]. The Hg sample was found in the body lotion sample because it was deliberately added because it served to whiten the skin in a relatively short time [11]. The results of quantitative analysis of body lotion samples show in Table 5.

Mercury in cosmetics is found in the form of mercury chloride (HgCl_2) and mercury amido chloride (HgNH_2Cl) compounds. The mechanism of action of mercury in whitening the skin varies. Mercury chloride in the skin will release hydrochloric acid which causes peeling of the epidermis layer. Whereas mercury amido chloride has the activity of inhibiting the work

of the enzyme tyrosinase which plays a role in the process of melanin [18].

Table 5. Quantitative test results using CV AAS

No	Sample Code	Concentration (ppb)
1	TB ₁	<0,0001
2	TB ₂	<0,0001
3	TB ₃	0,046
4	TB ₄	<0,0001
5	TB ₆	0,001
6	TB ₉	<0,0001
7	TB ₁₀	<0,0001
8	TB ₁₃	0,0040
9	B ₁	<0,0001
10	B ₂	<0,0001
11	B ₃	<0,0001

The formation of melanin is very important for the development of human skin color. Melanin is a dark brown pigment produced by melanocytes and stored in epidermal cells [17]. Melanin deficiency will cause easy UV exposure directly to human skin so that the skin becomes wrinkled, dull, dry, black spots arise to skin cancer [19].

Mercury with a concentration above the allowed quality standard on the body lotion is not safe to use. Hg metal that has been exposed to the body will affect the metabolic system. Hg metal will accumulate in the blood and cannot be excreted, so Hg settles in the kidney and affects its function. Hg metal is very toxic, blocking the action of enzymes and damaging cell wall membranes. This situation is caused by the ability of Hg to form strong bonds with sulfur groups contained in enzymes and cell walls [20].

Inorganic mercury in the human body will form complexes of Hg and glutathion in the liver and kidneys that contain the amino acid cysteine. Furthermore, Hg forms a complex of mercury glutathion or cysteine mercury. In addition, Hg metal can form complexes with bile and will be absorbed by the large intestine so that it affects the metabolic system in the body [21].

CONCLUSION

By The quantitative analysis shows from eleven samples analyzed with CVAAS only three samples (TB_3 , TB_6 , and TB_{13}) were positive for Hg. Based on the results of research that has been done it can be concluded that all samples analyzed are still below the quality standard set by BPOM.

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